(FILE 'MEDLINE, AGRICOLA, CANCERLIT, SCISEARCH, CAPLUS, EMBASE, BIOSIS, MEDICONF' ENTERED AT 15:36:36 ON 30 AUG 2002)

```
                DEL HIS
L1          170 S ENDOTHELIAL PROTEIN C RECEPTOR?
L2           76 DUP REM L1 (94 DUPLICATES REMOVED)
L3            5 S L2 AND (CONJUGAT? OR HYBRID? OR FUS? OR LINK?)
L4            5 SORT L3 PY
L5         4353 S (PROTEIN C) (L) (CONJUGAT? OR HYBRID? OR FUS? OR LINK?)
L6         3802 S (PROTEIN C) (S) (CONJUGAT? OR HYBRID? OR FUS? OR LINK?)
L7          670 S L6 AND (PROTEIN C (L) DNA)
L8          274 DUP REM L7 (396 DUPLICATES REMOVED)
L9          274 FOCUS L8 1-
L10         773 S L5 AND (PROTEIN C (L) DNA)
L11           6 FOCUS L10 1-6
L12         118 S L10 AND ANTIBOD?
L13         118 FOCUS L12 1-
L14         523 S L10 AND (PROTEIN C (S) CONJUGAT? OR HYBRID OR FUSION OR LINK?
L15           5 S L14 AND CONJUGATED
L16         207 DUP REM L14 (316 DUPLICATES REMOVED)
                E ESMON C?/AU
L17         345 S E6
L18        3802 S L6 AND PROTEIN (W)C
L19         247 S L17 AND (PROTEIN(W)C)
L20         179 DUP REM L19 (68 DUPLICATES REMOVED)
L21          42 S L20 AND ((ENDOTHELIAL PROTEIN C RECEPTOR) OR EPCR)
L22          42 SORT L21 PY
L23           1 S L22 AND FUSION
L24           7 S L22 AND (FUSION OR CONJUGATE? OR LINK? OR HYBRID)

=> d an ti so au ab pi l24 3 4 6 7

L24  ANSWER 3 OF 7  CAPLUS  COPYRIGHT 2002 ACS
AN   2000:172846  CAPLUS
DN   132:217958
TI   Diagnostic assays using soluble endothelial cell protein
     C/activated protein C receptor
SO   U.S., 24 pp., Division of U.S. Ser. 884,203.
     CODEN: USXXAM
IN   Esmon, Charles T.; Stearns-Kurosawa, Deborah J.; Kurosawa,
     Shinichiro
AB   Plasma EPCR has been isolated, characterized and shown to block
     cellular protein C activation and APC anticoagulant
     activity.  Plasma EPCR appears to be about 43,000 daltons and
     circulates at approx. 100 ng/mL (98.4.+-.27.8 ng/mL, n=22).  Plasma
     EPCR bound activated protein C with an
     affinity similar to that of recombinant sol. EPCR (Kdapp approx.
     30 nM), and inhibits both protein C activation on an
     endothelial cell line and APC anticoagulant activity in a one-stage factor
     Xa clotting assay.  Sol. plasma EPCR appears to attenuate the
     membrane-bound EPCR augmentation of protein C
     activation and the anticoagulant function of activated protein
     C.  Sol. EPCR has also been detected in urine.  Levels
     of sol. EPCR can rise in inflammatory disease assocd. with
     vascular injury and appear to be correlated with inflammation and disease
     states assocd. with abnormal coagulation.  Since EPCR expression
     is restricted to larger vessels and is usually neg. in capillaries, these
     observations provide a mechanism for analyzing injury/stimulation of large
     vessel endothelial cells.
     PATENT NO.        KIND  DATE          APPLICATION NO.  DATE
     --------------    ----  --------      --------------   --------
PI   US 6037450         A    20000314      US 1998-82021    19980520

L24  ANSWER 4 OF 7  CAPLUS  COPYRIGHT 2002 ACS
AN   2000:144771  CAPLUS
DN   132:176588
TI   Targeting of molecules to nuclei of cells of the large vessel endothelium
     using endothelial protein C receptor
     as a target
SO   PCT Int. Appl., 24 pp.
     CODEN: PIXXD2
```

IN **Esmon, Charles T.**; Xu, Jun

AB **Endothelial protein C receptor** (**EPCR**) is found primarily on endothelial cells of large vessels. **EPCR** translocates from the plasma membrane surface to the nucleus. Mols. which bind to **EPCR** can be carried from the plasma membrane surface to the nucleus. These mols. include antibodies to **EPCR** and activated **protein C**. **Protein C** , which also binds to **EPCR**, can be internalized by endothelial cells, but does not enter the nucleus. Thus, **EPCR** translocation from the plasma membrane to the nucleus provides a means of delivering nucleic acid such as DNA, proteins such as transcription factors, diagnostic agents or other types of drugs to the nucleus of endothelial cells, particularly those on large blood vessels. **Conjugates** of the materials to be delivered to the nucleus can be formed by ionic or covalent coupling. For example, proteins, including **fusion** proteins, can be directly **conjugated** to an anti-**EPCR** monoclonal antibody. Covalent attachment of pos. charged polymers, such as polylysine, to an anti-**EPCR** antibody allows nucleic acid to bind by ionic charges. Streptavidin and biotin can also be used to **conjugate** mols. to anti-**EPCR** antibodies. These **conjugated** antibodies are transported to the nucleus by **EPCR**. Examples demonstrate selective transport to the nucleus which is mediated by **EPCR**. Mols. transported include activated **protein C**, antibodies to **EPCR**, and streptavidin-biotin **conjugates**. Modification of anti-**EPCR** monoclonal antibodies by covalently coupling to polylysine allows binding of an expression vector to the modified antibody and translocation to the nucleus.

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2000010609	A1	20000302	WO 1999-US19480	19990825
W: AU, CA, JP				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
AU 9959013	A1	20000314	AU 1999-59013	19990825
EP 1107790	A1	20010620	EP 1999-946649	19990825
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2002523381	T2	20020730	JP 2000-565929	19990825

L24 ANSWER 6 OF 7 CAPLUS COPYRIGHT 2002 ACS

AN 1998:590692 CAPLUS

DN 129:213853

TI Diagnostic assays using soluble endothelial cell **protein C**/activated **protein C** receptor

SO U.S., 23 pp.
CODEN: USXXAM

IN **Esmon, Charles T.**; Stearns-Kurosawa, Deborah J.; Kurosawa, Shinichiro

AB Plasma endothelial cell **protein C**/activated **protein C** receptor (**EPCR**) has been isolated, characterized and shown to block cellular **protein C** activation and APC (activated **protein C**) anticoagulant activity. Plasma **EPCR** appears to be about 43,000 Da and circulates at approx. 100 ng/mL (98.4.+-.27.8 ng/mL, n=22). Plasma **EPCR** bound activated **protein C** with an affinity similar to that of recombinant sol. **EPCR** (Kdapp approx. 30 nM), and inhibits both **protein C** activation on an endothelial cell line and APC anticoagulant activity in a one-stage factor Xa clotting assay. Sol. plasma **EPCR** appears to attenuate the membrane-bound **EPCR** augmentation of **protein C** activation and the anticoagulant function of activated **protein C**. Sol. **EPCR** has also been detected in urine. Levels of sol. **EPCR** can rise in inflammatory disease assocd. with vascular injury and appear to be correlated with inflammation and disease states assocd. with abnormal coagulation. Since **EPCR** expression is restricted to larger vessels and is usually neg. in capillaries, these observations provide a mechanism for analyzing injury/stimulation of large vessel endothelial cells.

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE

PI	US 5804392	A	19980908	US 1997-884203	19970627
	WO 9900673	A1	19990107	WO 1998-US13385	19980626
	W: AU, CA, JP				
	RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
	AU 9882694	A1	19990119	AU 1998-82694	19980626
	AU 748199	B2	20020530		
	EP 991946	A1	20000412	EP 1998-932912	19980626
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
	JP 2001508876	T2	20010703	JP 1999-505810	19980626
	JP 2002193998	A2	20020710	JP 2001-365405	19980626

L24 ANSWER 7 OF 7 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:322310 BIOSIS
TI Identification of the **protein** C/activated **protein C** binding domain on the endothelial cell **protein C** receptor: Implications for a novel mode of ligand recognition by an MHC class 1-type receptor.
SO Blood, (November 16, 2000) Vol. 96, No. 11 Part 1, pp. 813a. print. Meeting Info.: 42nd Annual Meeting of the American Society of Hematology San Francisco, California, USA December 01-05, 2000 American Society of Hematology . ISSN: 0006-4971.
AU Liaw, Patricia C. Y.; Mather, Timothy (1); Oganesyan, Natalia (1); **Esmon, Charles T. (1)**
AB The endothelial cell **protein C** receptor (**EPCR**) is an endothelial cell-specific transmembrane protein that binds both **protein C** and activated **protein C** (APC). **EPCR** regulates the **protein C** anticoagulant pathway by binding **protein C** and augmenting **protein C** activation by the thrombin-thrombomodulin complex. **EPCR** is homologous to the MHC class 1/CD1 family, members of which contain a deep ligand binding groove formed by two antiparallel alpha-helices that sit upon an 8-stranded beta-sheet platform. In this study, we set out to identify residues that form the **protein C**/APC binding domain on human **EPCR**. Alanine mutagenesis and flow cytometric analysis of 293 cells transfected with **EPCR** variants identified ten residues that are involved in **protein C**/APC binding (R81, L82, V83, E86, R87, F146, Y154, T157, R158, and E160). Alanine substitutions at the four N-**linked** carbohydrate attachment sites of **EPCR** do not affect APC binding, suggesting that the carbohydrate moieties of **EPCR** are not important for ligand recognition. We next set out to map the epitopes for four anti-human **EPCR** monoclonal antibodies (mAbs), two of which block **EPCR**/F1-APC interactions (JRK 1494 and JRK 1535) whereas two do not (JRK 1500 and JRK 1513). The overall goal of the epitope-mapping studies is to see if the epitopes for the blocking mAbs co-localize with any of the 10 residues implicated in **protein C**/APC binding, thereby increasing our confidence in the definitive assignment of the ten residues in **protein C**/APC binding. Since these mAbs recognize human but not mouse **EPCR**, human-mouse chimeras were generated for use in gain-of-function epitope mapping strategy. Based on this approach, we found that five of the ten candidate residues for **protein C**/APC binding (R81, L82, V83, E86, R87) co-localize with the epitope for the blocking mAb JRK 1494. The remaining five candidate residues (F146, Y154, T157, R158, E160) co-localize with the epitope for JRK 1535, also a blocking mAb. **Protein C** activation studies on 293 cells that co-express **EPCR** variants and thrombomodulin demonstrate that **protein C** binding to **EPCR** is necessary for the **EPCR**-dependent enhancement in protein activation by the thrombin-thrombomodulin complex. 3-D molecular modeling of **EPCR** indicate that the **protein C**/APC binding candidate residues are located in the distal end of the two alpha-helical segments that form the putative ligand binding groove. Taken together, these studies have two important implications. First, our studies suggest that **EPCR** has exploited the MHC class 1 fold for an alternative and possibly novel mode of ligand recognition. Second, these studies are the

first to identify the **protein** C/APC binding region of **EPCR** and may provide useful information about molecular defects in **EPCR** that could contribute to cardiovascular disease susceptibility.

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(FILE 'MEDLINE, AGRICOLA, CANCERLIT, SCISEARCH, CAPLUS, EMBASE, BIOSIS, MEDICONF' ENTERED AT 15:36:36 ON 30 AUG 2002)

DEL HIS

L1 170 S ENDOTHELIAL PROTEIN C RECEPTOR?
L2 76 DUP REM L1 (94 DUPLICATES REMOVED)
L3 5 S L2 AND (CONJUGAT? OR HYBRID? OR FUS? OR LINK?)
L4 5 SORT L3 PY

=> d an ti so au ab pi l4 1-5

L4 ANSWER 1 OF 5 MEDLINE
AN 2000035741 MEDLINE
TI Organization and chromosomal localization of the human **endothelial protein C receptor** gene.
SO GENE, (1999 Oct 1) 238 (2) 367-73.
Journal code: 7706761. ISSN: 0378-1119.
AU Hayashi T; Nakamura H; Okada A; Takebayashi S; Wakita T; Yuasa H; Okumura K; Suzuki K
AB **Endothelial protein C receptor** (EPCR), present on endothelial cells of relatively large veins and arteries, plays a role in the enhancement of protein C activation by the thrombin-thrombomodulin complex. In the present study, we determined the organization and the complete nucleotide sequence of the human EPCR gene using polymerase chain reaction-direct sequencing method. The transcription initiation site of the EPCR gene was also determined by the cap site hunting method, using a cap site cDNA prepared from human placenta. The human EPCR gene spanned approx. 6 kb and was composed of four exons and three introns. All exon-intron boundaries agreed with the GT-AG rule. The 5'-flanking region (300 bp) of the EPCR gene contained a putative AP1-binding site, two Sp1-binding sites and two AP2-binding sites, but not definite TATAA or CCAAT sequences. Fluorescence in situ **hybridization** analysis showed that the EPCR gene is located in chromosome 20q11.2.

L4 ANSWER 2 OF 5 MEDLINE
AN 2000487988 MEDLINE
TI The soluble **endothelial protein C receptor** binds to activated neutrophils: involvement of proteinase-3 and CD11b/CD18.
SO JOURNAL OF IMMUNOLOGY, (2000 Oct 15) 165 (8) 4697-703.
Journal code: 2985117R. ISSN: 0022-1767.
AU Kurosawa S; Esmon C T; Stearns-Kurosawa D J
AB The protein C pathway is a primary regulator of blood coagulation and a critical component of the host response to inflammatory stimuli. The most recent member of this pathway is the **endothelial protein C receptor** (EPCR), a type I transmembrane protein with homology to CD1d/MHC class I proteins. EPCR accelerates formation of activated protein C, a potent anticoagulant and antiinflammatory agent. The current study demonstrates that soluble EPCR binds to PMA-activated neutrophils. Using affinity chromatography, binding studies with purified components, and/or blockade with specific Abs, it was found that soluble EPCR binds to proteinase-3 (PR3), a neutrophil granule proteinase. Furthermore, soluble EPCR binding to neutrophils was partially dependent on Mac-1 (CD11b/CD18), a beta(2) integrin involved in neutrophil signaling, and cell-cell adhesion events. PR3 is involved in multiple diverse processes, including hemopoietic proliferation, antibacterial activity, and autoimmune-mediated vasculitis. The observation that soluble EPCR binds to activated neutrophils via PR3 and a beta(2) integrin suggests that there may be a **link** between the protein C anticoagulant pathway and neutrophil functions.

L4 ANSWER 3 OF 5 MEDLINE
AN 2000239888 MEDLINE
TI Characterization and regulation of the 5'-flanking region of the murine **endothelial protein C receptor** gene.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (2000 Apr 28) 275 (17) 12481-8.
Journal code: 2985121R. ISSN: 0021-9258.
AU Gu J M; Fukudome K; Esmon C T
AB The protein C pathway plays a critical role in the negative regulation of blood coagulation. The nucleotide sequence of the murine

endothelial protein C receptor
(mEPCR) gene was determined for 8.8 kilobase pairs of the genomic structure and 3.4 kilobase pairs of the 5'-flanking region. RNase protection assay revealed six major transcription start sites clustered at -100 to -109 upstream of the translation initiation site. A series of 5'-promoter deletion fragments were **fused** to a luciferase reporter gene and transiently transfected into bovine aortic endothelium. Deletion of the sequence from -220 to -180 dramatically reduced luciferase expression in bovine aortic endothelial cells. This region of the murine **endothelial protein C receptor** gene contains one AP4 site and one SP1 site. Mutations in the core sequence of the AP4 and SP1 sites impaired both nuclear protein binding and luciferase expression. These results suggest important roles for AP4 and SP1 in the constitutive expression of mEPCR. A thrombin response element (CCCACCCC) was found to mediate the induction of mEPCR by thrombin in cell culture. Transgenic mice were developed expressing green fluorescent protein driven by the -350 to -1 or -1080 to -1 promoter. Thrombin up-regulated mEPCR and the transgene in vivo.

L4 ANSWER 4 OF 5 CAPLUS COPYRIGHT 2002 ACS
AN 2000:144771 CAPLUS
DN 132:176588
TI Targeting of molecules to nuclei of cells of the large vessel endothelium using **endothelial protein C receptor** as a target
SO PCT Int. Appl., 24 pp.
CODEN: PIXXD2
IN Esmon, Charles T.; Xu, Jun
AB **Endothelial protein C receptor** (EPCR) is found primarily on endothelial cells of large vessels. EPCR translocates from the plasma membrane surface to the nucleus. Mols. which bind to EPCR can be carried from the plasma membrane surface to the nucleus. These mols. include antibodies to EPCR and activated protein C. Protein C, which also binds to EPCR, can be internalized by endothelial cells, but does not enter the nucleus. Thus, EPCR translocation from the plasma membrane to the nucleus provides a means of delivering nucleic acid such as DNA, proteins such as transcription factors, diagnostic agents or other types of drugs to the nucleus of endothelial cells, particularly those on large blood vessels. **Conjugates** of the materials to be delivered to the nucleus can be formed by ionic or covalent coupling. For example, proteins, including **fusion** proteins, can be directly **conjugated** to an anti-EPCR monoclonal antibody. Covalent attachment of pos. charged polymers, such as polylysine, to an anti-EPCR antibody allows nucleic acid to bind by ionic charges. Streptavidin and biotin can also be used to **conjugate** mols. to anti-EPCR antibodies. These **conjugated** antibodies are transported to the nucleus by EPCR. Examples demonstrate selective transport to the nucleus which is mediated by EPCR. Mols. transported include activated protein C, antibodies to EPCR, and streptavidin-biotin **conjugates**. Modification of anti-EPCR monoclonal antibodies by covalently coupling to polylysine allows binding of an expression vector to the modified antibody and translocation to the nucleus.

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2000010609	A1	20000302	WO 1999-US19480	19990825
	W: AU, CA, JP				
	RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
	AU 9959013	A1	20000314	AU 1999-59013	19990825
	EP 1107790	A1	20010620	EP 1999-946649	19990825
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
	JP 2002523381	T2	20020730	JP 2000-565929	19990825

L4 ANSWER 5 OF 5 MEDLINE
AN 2001413411 MEDLINE
TI Staging of the pathophysiologic responses of the primate microvasculature to Escherichia coli and endotoxin: examination of the elements of the compensated response and their **links** to the corresponding uncompensated lethal variants.

SO CRITICAL CARE MEDICINE, (2001 Jul) 29 (7 Suppl) S78-89. Ref: 125
Journal code: 0355501. ISSN: 0090-3493.

AU Taylor F B Jr

AB OBJECTIVE: Review of primate studies of Escherichia coli sepsis and endotoxemia with a reexamination of the rationale for diagnosis and treatment of these multistage disorders. SETTING: Animal research and intensive care units in a university medical school. SUBJECTS: Cyanocephalus baboons (E. coli) and normal human subjects (endotoxin). INTERVENTIONS: Baboon studies: anti-tissue factor, protein C, **endothelial protein C receptor**, and anti-tumor necrosis factor antibodies, and active site inhibited factor recombinant VIIa and factor Xa. RESULTS AND CONCLUSIONS: This review concerns the primate microvascular endothelial response to inflammatory and hemostatic stress. Studies of the impact of inflammatory and hemostatic stress on this microvasculature have fallen into four categories. First, studies of pure hemostatic stress using factor Xa phospholipid vesicles showed that blockade of protein C as well as protein C plus tissue plasminogen activator produced a severe but transient consumptive and a lethal thrombotic coagulopathy, respectively. These studies showed that the protein C and fibrinolytic systems can work in tandem to regulate even a severe response if the endothelium is not rendered dysfunctional by metabolic or inflammatory factors. Second, studies of compensated (nonlethal) inflammatory stress using E. coli or endotoxin in baboon and human subjects showed that even under minimal stress in which there is no evidence of overt disseminated intravascular coagulation, injury of the endothelium and activation of neutrophils and hemostatic factors are closely associated. This showed that molecular markers of hemostatic activity could be used to detect microvascular endothelial stress (nonovert disseminated intravascular coagulation) in patients who are compensated but at risk. These studies also showed that the compensated response to inflammatory stress could exhibit two stages, each with its unique inflammatory and hemostatic response signature. The first is driven by vasoactive peptides, cytokines, and thrombin, followed 12 to 14 hrs later by a second stage driven by C-reactive protein/complement complexes, tissue factor, and plasminogen activator inhibitor 1 secondary to oxidative stress after reperfusion. Third, studies of uncompensated (lethal) inflammatory stress using E. coli showed that irreversible thrombosis of the microvasculature was not a **link** in the lethal chain of events even though inhibition of components of the protein C network (protein C and **endothelial protein C receptor**) converted compensated responses to sublethal E. coli into uncompensated lethal responses. Fourth, these studies also showed that there were variants of the lethal response ranging from capillary leak and shock to recurrent sustained inflammatory disorders. We believe that each of these variants arises from their sublethal counterparts, depending on underlying or modulating host factors operating at the time of challenge. Such underlying conditions range from preexisting microvascular ischemia, reperfusion, and oxidative stress to alteration or reprogramming of monocyte/macrophage responses (tolerance to hyperresponsiveness). Characterization of these underlying conditions in patients who are at risk should aid in identifying and optimizing management of these variants.

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(FILE 'MEDLINE, AGRICOLA, CANCERLIT, SCISEARCH, CAPLUS, EMBASE, BIOSIS, MEDICONF' ENTERED AT 15:36:36 ON 30 AUG 2002)
DEL HIS
L1 170 S ENDOTHELIAL PROTEIN C RECEPTOR?
L2 76 DUP REM L1 (94 DUPLICATES REMOVED)
L3 5 S L2 AND (CONJUGAT? OR HYBRID? OR FUS? OR LINK?)
L4 5 SORT L3 PY

=> d all l4 4

L4 ANSWER 4 OF 5 CAPLUS COPYRIGHT 2002 ACS
AN 2000:144771 CAPLUS
DN 132:176588
TI Targeting of molecules to nuclei of cells of the large vessel endothelium using **endothelial protein C receptor** as a target
IN Esmon, Charles T.; Xu, Jun
PA Oklahoma Medical Research Foundation, USA
SO PCT Int. Appl., 24 pp.
CODEN: PIXXD2
DT Patent
LA English
IC ICM A61K045-06
CC 3-1 (Biochemical Genetics)
Section cross-reference(s): 9
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2000010609	A1	20000302	WO 1999-US19480	19990825
	W: AU, CA, JP				
	RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
	AU 9959013	A1	20000314	AU 1999-59013	19990825
	EP 1107790	A1	20010620	EP 1999-946649	19990825
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
	JP 2002523381	T2	20020730	JP 2000-565929	19990825
PRAI	US 1998-139425	A	19980825		
	WO 1999-US19480	W	19990825		

AB **Endothelial protein C receptor** (EPCR) is found primarily on endothelial cells of large vessels. EPCR translocates from the plasma membrane surface to the nucleus. Mols. which bind to EPCR can be carried from the plasma membrane surface to the nucleus. These mols. include antibodies to EPCR and activated protein C. Protein C, which also binds to EPCR, can be internalized by endothelial cells, but does not enter the nucleus. Thus, EPCR translocation from the plasma membrane to the nucleus provides a means of delivering nucleic acid such as DNA, proteins such as transcription factors, diagnostic agents or other types of drugs to the nucleus of endothelial cells, particularly those on large blood vessels. **Conjugates** of the materials to be delivered to the nucleus can be formed by ionic or covalent coupling. For example, proteins, including **fusion** proteins, can be directly **conjugated** to an anti-EPCR monoclonal antibody. Covalent attachment of pos. charged polymers, such as polylysine, to an anti-EPCR antibody allows nucleic acid to bind by ionic charges. Streptavidin and biotin can also be used to **conjugate** mols. to anti-EPCR antibodies. These **conjugated** antibodies are transported to the nucleus by EPCR. Examples demonstrate selective transport to the nucleus which is mediated by EPCR. Mols. transported include activated protein C, antibodies to EPCR, and streptavidin-biotin **conjugates**. Modification of anti-EPCR monoclonal antibodies by covalently coupling to polylysine allows binding of an expression vector to the modified antibody and translocation to the nucleus.

ST endothelium large vessel targetting **endothelial protein C receptor**; transformation vascular endothelium targetting **endothelial protein C receptor**

IT Polyelectrolytes

(cationic, in binding substances to ligands for **endothelial protein C receptor**; targeting of mols. to nuclei of cells of large vessel endothelium using **endothelial protein C receptor** as target)

IT Antibodies
RL: BUU (Biological use, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(chimeric, in binding substances to ligands for **endothelial protein C receptor**; targeting of mols. to nuclei of cells of large vessel endothelium using **endothelial protein C receptor** as target)

IT Antisense DNA
Proteins, general, biological studies
Ribozymes
Transcription factors
RL: BUU (Biological use, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(delivery to large vessel endothelial cells of; targeting of mols. to nuclei of cells of large vessel endothelium using **endothelial protein C receptor** as target)

IT Receptors
RL: BUU (Biological use, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(endothelial protein C (EPCR); targeting of mols. to nuclei of cells of large vessel endothelium using **endothelial protein C receptor** as target)

IT Blood vessel
(endothelium; targeting of mols. to nuclei of cells of large vessel endothelium using **endothelial protein C receptor** as target)

IT Drug delivery systems
(for large vessel endothelial cells; targeting of mols. to nuclei of cells of large vessel endothelium using **endothelial protein C receptor** as target)

IT RNA
RL: BUU (Biological use, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(guide, delivery to large vessel endothelial cells of; targeting of mols. to nuclei of cells of large vessel endothelium using **endothelial protein C receptor** as target)

IT Antibodies
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(monoclonal, to EPCR, in targetted delivery of materials; targeting of mols. to nuclei of cells of large vessel endothelium using **endothelial protein C receptor** as target)

IT Transformation, genetic
(of vascular endothelial cells; targeting of mols. to nuclei of cells of large vessel endothelium using **endothelial protein C receptor** as target)

IT Blood vessel
(targeting of mols. to nuclei of cells of large vessel endothelium using **endothelial protein C receptor** as target)

IT Antibodies
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(to EPCR, in targetted delivery of materials; targeting of mols. to nuclei of cells of large vessel endothelium using **endothelial protein C receptor** as target)

IT Oligonucleotides
RL: BUU (Biological use, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(triple helix-forming, delivery to large vessel endothelial cells of; targeting of mols. to nuclei of cells of large vessel endothelium using **endothelial protein C receptor** as target)

IT Biological transport

(uptake, of complexes bound to **endothelial protein C receptor**; targeting of mols. to nuclei of cells of large vessel endothelium using **endothelial protein C receptor** as target)

IT 60202-16-6D, Protein C, complexes
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(delivery to large vessel endothelial cells of; targeting of mols. to nuclei of cells of large vessel endothelium using **endothelial protein C receptor** as target)

IT 25104-18-1D, Polylysine, **conjugates** with proteins, complexes with nucleic acids 38000-06-5D, Polylysine, **conjugates** with proteins, complexes with nucleic acids
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(for targetting EPCR; targeting of mols. to nuclei of cells of large vessel endothelium using **endothelial protein C receptor** as target)

IT 58-85-5, Biotin 9013-20-1, Streptavidin
RL: BUU (Biological use, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(in binding substances to ligands for **endothelial protein C receptor**; targeting of mols. to nuclei of cells of large vessel endothelium using **endothelial protein C receptor** as target)

RE.CNT 9 THERE ARE 9 CITED REFERENCES AVAILABLE FOR THIS RECORD
RE
(1) American Nat Red Cross; WO 8902747 A 1989 CAPLUS
(2) Baxter Int; WO 9008556 A 1990 CAPLUS
(3) Immuno AG; EP 0687687 A 1995 CAPLUS
(4) Jakubowski, J; WO 9855142 A 1998 CAPLUS
(5) Oklahoma Med Res Found; WO 9605303 A 1996 CAPLUS
(6) Oklahoma Med Res Found; WO 9820041 A 1998 CAPLUS
(7) Oklahoma Med Res Found; WO 9900673 A 1999 CAPLUS
(8) Scripps Clinic Res; EP 0318201 A 1989 CAPLUS
(9) Taylor, F; US 5009889 A 1991 CAPLUS

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L9 ANSWER 2 OF 274 CAPLUS COPYRIGHT 2002 ACS
AN 1989:451707 CAPLUS
DN 111:51707
TI Recombinant **hybrid protein C** with altered Gla domain for use as anticoagulant
SO Eur. Pat. Appl., 23 pp.
CODEN: EPXXDW
IN Iwasaki, Wakako; Takahashi, Mikiko; Hashimoto, Tamotsu
AB Recombinant, **hybrid**, human **protein C** with the Gla domain (residues 1-43) replaced by the corresponding Gla domain of prothrombin, Factor VII, Factor IX, or Factor X is prepd. These hybrid proteins have altered anticoagulant activities. Plasmid pCs4, contg. **protein C**-encoding **DNA**, was digested with SalI and the SaLI fragment was replaced with a synthetic fragment encoding the leader sequence and first 43 amino acids of Factor X. The resulting plasmid pCs6 was used to prep. a mammalian cell expression vector. CHO cells transfected with this vector produced **hybrid protein C**, which was purified by the method of Kisiel, and tested for biol. activity. The recombinant protein was more potent than native **protein C** in inactivation of Factor Va and equally as effective as the native protein in inactivation of Factor VIIIc.

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI EP 296413	A2	19881228	EP 1988-109186	19880609
R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE				
FI 8802746	A	19881213	FI 1988-2746	19880609
JP 01085096	A2	19890330	JP 1988-140558	19880609
JP 2799316	B2	19980917		
DK 8803172	A	19881213	DK 1988-3172	19880610
AU 8817565	A1	19881215	AU 1988-17565	19880610

L9 ANSWER 4 OF 274 CAPLUS COPYRIGHT 2002 ACS
AN 2002:314794 CAPLUS
DN 136:345751
TI Protein C or activated protein C-like molecules
SO PCT Int. Appl., 92 pp.
CODEN: PIXXD2
IN Andersen, Kim Vilbour; Pedersen, Anders Hjelholt; Freskgaard, Per Ola
AB The present invention relates to novel **conjugates** between polypeptide variants of **protein C** and a non-polypeptide moiety, such as PEG or sugar moieties. In particular, the present invention provides novel **protein C conjugates** having an increased resistance to inactivation by e.g. human plasma and .alpha.1-antitrypsin. Consequently, such conjugates have an increased in vivo half-life. Preferred examples include **protein C conjugates**, wherein at least one addnl. in vivo N-glycosylation site has been introduced. The conjugates of the invention are useful for treating a variety of diseases, including septic shock.

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2002032461	A2	20020425	WO 2001-DK679	20011015

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

L13 ANSWER 6 OF 118 CAPLUS COPYRIGHT 2002 ACS
AN 1984:623801 CAPLUS
DN 101:223801
TI Characterization of a cDNA coding for human protein C
SO Proc. Natl. Acad. Sci. U. S. A. (1984), 81(15), 4766-70
CODEN: PNASA6; ISSN: 0027-8424
AU Foster, Donald; Davie, Earl W.
AB **Protein C** is a precursor to a serine protease that is present in mammalian plasma. In its activated form (blood coagulation factor XIVa [42617-41-4]) it readily inactivates factor Va and factor VIIIa, 2 proteins that participate as cofactors in the blood coagulation cascade. A .lambda.gt11 library contg. cDNA inserts prepd. from human liver mRNA was screened with an **antibody** to human **protein C**. Seven pos. clones were isolated from 2 .times. 106 phage and were plaque-purified. The cDNA inserts of 2 of these phage were sequenced and shown to code for human **protein C**. Each cDNA insert coded for a portion of the light chain of the mol., a connecting region, the heavy chain, a stop codon, a 3'-noncoding region, and a poly(A) tail. The length of the noncoding sequence on the 3' end differed in the 2 clones, but each contained a processing or polyadenylylation signal that was followed by a poly(A) tail. The amino acid sequence, as detd. from the cDNA, indicates that **protein C** is synthesized as a single-chain polypeptide contg. the light chain and the heavy chain connected by a dipeptide of Lys-Arg. The single-chain mol. is then converted to the light and heavy chains by cleavage of .gtoreq.2 internal peptide bonds. In plasma, the heavy and light chains of **protein C** are **linked** together by a disulfide bond. The amino acid sequence of human **protein C** shows a high degree of homol. with that of the bovine mol. The **DNA** sequence coding for the catalytic region near the active site serine in human **protein C** also showed a high degree of **DNA** and amino acid sequence identity with prothrombin, factor IX, and factor X, 3 of the other vitamin K-dependent serine proteases that are present in plasma.

=> d an ti so au ab pi l13 3

L13 ANSWER 3 OF 118 CAPLUS COPYRIGHT 2002 ACS
AN 2000:144771 CAPLUS
DN 132:176588
TI Targeting of molecules to nuclei of cells of the large vessel endothelium using endothelial protein C receptor as a target
SO PCT Int. Appl., 24 pp.
CODEN: PIXXD2
IN Esmon, Charles T.; Xu, Jun
AB Endothelial **protein C** receptor (EPCR) is found primarily on endothelial cells of large vessels. EPCR translocates from the plasma membrane surface to the nucleus. Mols. which bind to EPCR can be carried from the plasma membrane surface to the nucleus. These mols. include **antibodies** to EPCR and activated **protein C**. **Protein C**, which also binds to EPCR, can be internalized by endothelial cells, but does not enter the nucleus. Thus, EPCR translocation from the plasma membrane to the nucleus provides a means of delivering nucleic acid such as **DNA**, proteins such as transcription factors, diagnostic agents or other types of drugs to the nucleus of endothelial cells, particularly those on large blood vessels. **Conjugates** of the materials to be delivered to the nucleus can be formed by ionic or covalent coupling. For example, proteins, including **fusion** proteins, can be directly **conjugated** to an anti-EPCR monoclonal **antibody**. Covalent attachment of pos. charged polymers, such as polylysine, to an anti-EPCR **antibody** allows nucleic acid to bind by ionic charges. Streptavidin and biotin can also be used to **conjugate** mols. to anti-EPCR **antibodies**. These **conjugated antibodies** are transported to the nucleus by EPCR. Examples demonstrate selective transport to the nucleus which is mediated by EPCR. Mols. transported include activated **protein C**, **antibodies** to EPCR, and streptavidin-biotin **conjugates**. Modification of anti-EPCR monoclonal **antibodies** by covalently coupling to polylysine allows binding of an expression vector to the modified **antibody** and translocation to the nucleus.

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2000010609	A1	20000302	WO 1999-US19480	19990825
W: AU, CA, JP				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
AU 9959013	A1	20000314	AU 1999-59013	19990825
EP 1107790	A1	20010620	EP 1999-946649	19990825
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2002523381	T2	20020730	JP 2000-565929	19990825

L22 ANSWER 8 OF 42 CAPLUS COPYRIGHT 2002 ACS
AN 1997:778163 CAPLUS
DN 128:73227
TI Human **protein C** receptor is present primarily on endothelium of large blood vessels: implications for the control of the **protein C** pathway
SO Circulation (1997), 96(10), 3633-3640
CODEN: CIRCAZ; ISSN: 0009-7322
AU Laszik, Zoltan; Mitro, Alexander; Taylor, Fletcher B., Jr.; Ferrell, Gary; **Esmon, Charles T.**
AB The **protein C** anticoagulant pathway is crit. to the control of hemostasis. Thrombomodulin and a newly identified receptor for **protein C**/activated **protein C**, **EPCR**, are both present on endothelium. **EPCR** augments activation of **protein C** by the thrombin-thrombomodulin complex. To gain a better understanding of the relationship between thrombomodulin and **EPCR**, we compared the cellular specificity and tissue distributions of these two receptors by using immunohistochem. **EPCR** expression was detected almost exclusively on endothelium in human and baboon tissues. In most organs, **EPCR** was expressed relatively intensely on the endothelium of all arteries and veins, most arterioles, and some postcapillary venules. **EPCR** staining was usually neg. on capillary endothelial cells. In contrast, thrombomodulin was detected at high concns. in both large vessels and capillary endothelium. Both thrombomodulin and **EPCR** were expressed poorly on brain capillaries. The liver sinusoids were the only capillaries in which **EPCR** was expressed at moderate levels and thrombomodulin was low. **EPCR** and thrombomodulin were both expressed on the endothelium of vasa recta in the renal medulla, the lymph node subcapsular and medullary sinuses, and some capillaries within the adrenal gland. Even in these organs the majority of capillaries were **EPCR** neg. or stained weakly. These studies suggest that **EPCR** may be important in enhancing **protein C** activation on large vessels. The presence of high levels of **EPCR** on arterial vessels may help explain why partial **protein C** deficiency is a weak risk factor for arterial thrombosis.

L22 ANSWER 6 OF 42 CAPLUS COPYRIGHT 2002 ACS
AN 1996:321402 CAPLUS
DN 125:1378
TI Cloning and regulation of an endothelial cell **protein C** receptor and use for inflammation regulation
SO PCT Int. Appl., 57 pp.
CODEN: PIXXD2
IN Fukudome, Kenji; **Esmon, Charles T.**
AB Human **protein C** and activated **protein C** were shown to bind to endothelium specifically, selectively and saturably (Kd=30 nM, 7000 sites per cell) and in Ca2+ dependent fashion. Expression cloning revealed a 1.3 kb CDNA that coded for a novel type I transmembrane glycoprotein capable of binding **protein C**. This protein appears to be a member of the CD1/MHC superfamily. Like thrombomodulin, the receptor involved in **protein C** activation, the endothelial cell **protein C** receptor (**EPCR**) function and message are both down regulated by exposure of endothelium to TNF. Identification of **EPCR** as a member of the CD1/MHC superfamily provides insights into the role of **protein C** in regulating the inflammatory response, and detn. of methods for pharmaceutical use in manipulating the inflammatory response.

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9605303	A1	19960222	WO 1995-US9636	19950809
W: AU, CA, JP				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
US 5695993	A	19971209	US 1994-289699	19940812
CA 2199821	AA	19960222	CA 1995-2199821	19950809
AU 9532723	A1	19960307	AU 1995-32723	19950809
AU 707349	B2	19990708		
EP 777731	A1	19970611	EP 1995-929335	19950809
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE				
US 5852171	A	19981222	US 1997-878283	19970618
US 6399064	B1	20020604	US 1998-182616	19981029

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     (FILE 'MEDLINE, AGRICOLA, CANCERLIT, SCISEARCH, CAPLUS, EMBASE, BIOSIS,
     MEDICONF' ENTERED AT 15:36:36 ON 30 AUG 2002)
                DEL HIS
L1           170 S ENDOTHELIAL PROTEIN C RECEPTOR?
L2            76 DUP REM L1 (94 DUPLICATES REMOVED)
L3             5 S L2 AND (CONJUGAT? OR HYBRID? OR FUS? OR LINK?)
L4             5 SORT L3 PY
L5          4353 S (PROTEIN C) (L) (CONJUGAT? OR HYBRID? OR FUS? OR LINK?)
L6          3802 S (PROTEIN C) (S) (CONJUGAT? OR HYBRID? OR FUS? OR LINK?)
L7           670 S L6 AND (PROTEIN C (L) DNA)
L8           274 DUP REM L7 (396 DUPLICATES REMOVED)
L9           274 FOCUS L8 1-
L10          773 S L5 AND (PROTEIN C (L) DNA)
L11            6 FOCUS L10 1-6
L12          118 S L10 AND ANTIBOD?
L13          118 FOCUS L12 1-
L14          523 S L10 AND (PROTEIN C (S) CONJUGAT? OR HYBRID OR FUSION OR LINK?
L15            5 S L14 AND CONJUGATED
L16          207 DUP REM L14 (316 DUPLICATES REMOVED)
                E ESMON C?/AU
L17          345 S E6
L18         3802 S L6 AND PROTEIN (W)C
L19          247 S L17 AND (PROTEIN(W)C)
L20          179 DUP REM L19 (68 DUPLICATES REMOVED)
L21           42 S L20 AND ((ENDOTHELIAL PROTEIN C RECEPTOR) OR EPCR)
L22           42 SORT L21 PY
L23            1 S L22 AND FUSION
L24            7 S L22 AND (FUSION OR CONJUGATE? OR LINK? OR HYBRID)
                E FOSTER DONALD?/AU
                E FOSTER D?/AU
                E FOSTER DON?/AU
L25          143 S E6
L26            0 S L25 AND L1
L27           24 S L25 AND (PROTEIN C)
L28           20 DUP REM L27 (4 DUPLICATES REMOVED)
L29           20 SORT L28 PY

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L29 ANSWER 6 OF 20 CAPLUS COPYRIGHT 2002 ACS
AN 1988:606245 CAPLUS
DN 109:206245
TI Sequencing of gene and cDNA for human **protein C** and recombinant production of **protein C** and derivatives
SO Eur. Pat. Appl., 35 pp.
CODEN: EPXXDW
IN **Foster, Donald C.**; Murray, Mark J.; Berkner, Kathleen L.
AB The gene for human **protein C** is sequenced. Vectors contg. genomic or cDNA sequences encoding **protein C** or **protein C** derivs. are constructed for expression in animal cells. Plasmid pPC829 was constructed contg. the cDNA sequence for **protein C** lacking the region coding for the activation peptide. BHK cells transformed with the plasmid produced activated **protein C** in the culture media.

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI EP 266190	A2	19880504	EP 1987-309528	19871028
EP 266190	A3	19891025		
EP 266190	B1	19930623		
R: AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE				
US 4959318	A	19900925	US 1986-924462	19861029
NO 8704495	A	19880502	NO 1987-4495	19871028
NO 173741	B	19931018		
NO 173741	C	19940126		
AT 90966	E	19930715	AT 1987-309528	19871028
DK 8705670	A	19880430	DK 1987-5670	19871029
JP 01085084	A2	19890330	JP 1987-271959	19871029
JP 2561677	B2	19961211		
KR 9702166	B1	19970224	KR 1987-11997	19871029
CA 1340263	A1	19981215	CA 1987-550620	19871029
US 5516650	A	19960514	US 1994-225253	19940408
JP 08252094	A2	19961001	JP 1996-96200	19960326
JP 2922461	B2	19990726		

L29 ANSWER 1 OF 20 CAPLUS COPYRIGHT 2002 ACS
AN 1985:536271 CAPLUS
DN 103:136271
TI The nucleotide sequence of the gene for human **protein C**
SO Proc. Natl. Acad. Sci. U. S. A. (1985), 82(14), 4673-7
CODEN: PNASA6; ISSN: 0027-8424
AU **Foster, Donald C.**; Yoshitake, Shinji; Davie, Earl W.
AB A human genomic DNA library was screened for the gene for **protein C** by using a cDNA probe coding for the human protein. Three different overlapping .lambda. Charon 4A phage were isolated that contain inserts for the gene for a **protein C**. The complete sequence of the gene was detd. by the dideoxy method and shown to span .apprx.11 kilobases of DNA. The coding and 3' noncoding portion of the gene consists of 8 exons and 7 introns. The 8 exons code for a preproleader sequence of 42 amino acids, a light chain of 155 amino acids, a connecting dipeptide of Lys-Arg, and a heavy chain of 262 amino acids. The preproleader sequence and the connecting dipeptide are removed during processing, resulting in the mature protein composed of a heavy and a light chain held together by a disulfide bond. The heavy chain also contains the catalytic region for the serine protease. Two Alu sequences and 2 homologous repeats of .apprx.160 nucleotides were found in intron E. The 7 introns in the gene for **protein C** are located in essentially the same positions in the amino acid sequence as the 7 introns in the gene for human blood coagulation factor IX [9001-28-9], while the 1st 3 introns in **protein C** are located in the same positions as the 1st 3 in the gene for human prothrombin.

L29 ANSWER 4 OF 20 CAPLUS COPYRIGHT 2002 ACS
AN 1987:593361 CAPLUS
DN 107:193361
TI Propeptide of human **protein C** is necessary for .gamma.-carboxylation

SO Biochemistry (1987), 26(22), 7003-11
CODEN: BICHAW; ISSN: 0006-2960

AU **Foster, Donald C.**; Rudinski, Mark S.; Schach, Barbara G.; Berkner, Kathleen L.; Kumar, A. Ashok; Hagen, Frederick S.; Sprecher, Cindy A.; Insley, Margaret Y.; Davie, Earl W.

AB Mutants contg. deletions in the propeptide region of the cDNA for human **protein C** were constructed, and the cDNAs were then expressed in mammalian cell culture. These deletions included the removal of 4, 9, 12, 15, 16, or 17 amino acids comprising the C-terminal end of the leader sequence of 42 amino acids. The mutant proteins were then examd. by Western blotting, Ba citrate adsorption and pptn., amino acid sequence anal., and biol. activity, and compared with the native protein present in normal plasma. **Protein C** is readily synthesized in mammalian cell cultures, processed, and secreted as a 2-chain mol. with biol. activity. Furthermore, the pre portion or signal sequence in human **protein C** is 18 amino acids in length, and the pro portion of the leader sequence is 24 amino acids in length. Also, during biosynthesis and secretion, the N-terminal region of the propeptide (residues from about -12 through -17) is important for vitamin K-dependent carboxylation of **protein C**, whereas the C-terminal portion of the propeptide (residues -1 through -4) is important for the removal of the pro leader sequence by proteolytic processing.

L29 ANSWER 5 OF 20 CAPLUS COPYRIGHT 2002 ACS
AN 1987:548603 CAPLUS
DN 107:148603
TI Cloning and expression of human **protein C** cDNA
SO Eur. Pat. Appl., 52 pp.
CODEN: EPXXDW

IN Murray, Mark J.; Berkner, Kathleen L.; **Foster, Donald C.**; Davie, Earl W.

AB The cDNA for human **protein C** is sequenced, cloned, and expressed in mammalian cells. Plasmid pMMC contg. intron-free cDNA encoding human **protein C** was constructed from a no. of pos. clones isolated by screening a cDNA library constructed by using human liver mRNA. The cDNA in pMMC was inserted in plasmid pD7 (contg. the SV40 enhancer and the adenovirus-2 major late promoter and tripartite leader sequence) to create the expression plasmid pD7C. Baby hamster kidney cells transfected with pD7C secreted **protein C** into the medium, which was confirmed by ELISA and activity assay.

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	EP 215548	A1	19870325	EP 1986-304970	19860626
	EP 215548	B1	19930818		
	EP 215548	B2	19980107		
	R: AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE				
	US 4968626	A	19901106	US 1985-766109	19850815
	AT 93272	E	19930915	AT 1986-304970	19860626
	DK 8603085	A	19861228	DK 1986-3085	19860627
	NO 8602601	A	19861229	NO 1986-2601	19860627
	JP 62111690	A2	19870522	JP 1986-151303	19860627
	JP 2614848	B2	19970528		
	JP 09107976	A2	19970428	JP 1996-179612	19860627
	US 5073609	A	19911217	US 1989-375260	19890629
	US 5302529	A	19940412	US 1990-512961	19900423
	US 5516650	A	19960514	US 1994-225253	19940408

L29 ANSWER 6 OF 20 CAPLUS COPYRIGHT 2002 ACS
AN 1988:606245 CAPLUS
DN 109:206245
TI Sequencing of gene and cDNA for human **protein C** and recombinant production of **protein C** and derivatives
SO Eur. Pat. Appl., 35 pp.
CODEN: EPXXDW

IN **Foster, Donald C.**; Murray, Mark J.; Berkner, Kathleen L.

AB The gene for human **protein C** is sequenced. Vectors contg. genomic or cDNA sequences encoding **protein C** or **protein C** derivs. are constructed for expression in animal cells. Plasmid pPC829 was constructed contg. the cDNA sequence for

protein C lacking the region coding for the activation peptide. BHK cells transformed with the plasmid produced activated **protein C** in the culture media.

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	EP 266190	A2	19880504	EP 1987-309528	19871028
	EP 266190	A3	19891025		
	EP 266190	B1	19930623		
	R: AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE				
	US 4959318	A	19900925	US 1986-924462	19861029
	NO 8704495	A	19880502	NO 1987-4495	19871028
	NO 173741	B	19931018		
	NO 173741	C	19940126		
	AT 90966	E	19930715	AT 1987-309528	19871028
	DK 8705670	A	19880430	DK 1987-5670	19871029
	JP 01085084	A2	19890330	JP 1987-271959	19871029
	JP 2561677	B2	19961211		
	KR 9702166	B1	19970224	KR 1987-11997	19871029
	CA 1340263	A1	19981215	CA 1987-550620	19871029
	US 5516650	A	19960514	US 1994-225253	19940408
	JP 08252094	A2	19961001	JP 1996-96200	19960326
	JP 2922461	B2	19990726		

L29 ANSWER 7 OF 20 CAPLUS COPYRIGHT 2002 ACS
AN 1990:422235 CAPLUS
DN 113:22235
TI Improved manufacture of recombinant proteins in transformed animal cells by introduction of a gene for an activating protease or a protease inhibitor
SO Eur. Pat. Appl., 41 pp.
CODEN: EPXXDW
IN Mulvihill, Eileen R.; Berkner, Kathleen L.; **Foster, Donald C.**; Kumar, Ashok A.; Mackay, Vivian L.; Parker, Gary E.
AB The manuf. of proteins that need proteolytic processing for full activity is made more efficient by introduction of a gene encoding an appropriate processing protease into the producing cells. Similarly, introduction of specific protease inhibitors also improves efficiency. A cDNA clone for **protein C** was introduced into BHK cells and the content of the two-chain form of the enzyme produced by these cells or by cells transformed with a factor contg. a gene for the yeast processing protease KEX2 and the **protein C** cDNA. The control cells produced **protein C** as 70% single-chain form. The cells carrying the KEX2 gene produced 95% two-chain form. Modification of the KEX2 processing site of **protein C** by site directed mutagenesis resulted in manuf. of the protein at levels of 0.5-2.2 pg/cell/day.

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	EP 319944	A2	19890614	EP 1988-120423	19881207
	EP 319944	A3	19900516		
	EP 319944	B1	19940921		
	R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE				
	CA 1340740	A1	19990914	CA 1988-584774	19881202
	FI 8805679	A	19890609	FI 1988-5679	19881207
	ES 2059478	T3	19941116	ES 1988-120423	19881207
	AU 8826706	A1	19890608	AU 1988-26706	19881208
	DK 8806843	A	19890811	DK 1988-6843	19881208
	JP 02002338	A2	19900108	JP 1988-308983	19881208
	AU 9214926	A1	19921029	AU 1992-14926	19920416
	AU 651680	B2	19940728		
	US 5516650	A	19960514	US 1994-225253	19940408
	US 5648254	A	19970715	US 1994-275076	19940714

L29 ANSWER 9 OF 20 CAPLUS COPYRIGHT 2002 ACS
AN 1992:1746 CAPLUS
DN 116:1746
TI Manufacture of vitamin K-dependent proteins with heterologous phospholipid binding domains
SO PCT Int. Appl., 57 pp.
CODEN: PIXXD2

IN **Foster, Donald C.**

AB Vitamin K-dependent proteins, e.g. blood-coagulation factors, in which the phospholipid-binding gla domain is replaced by a corresponding domain from a vitamin K-independent protein are manufd. by expression of the gene in animal cell culture. A chimeric gene encoding a fusion protein of **protein C** lacking the gla domain and the placental anticoagulant protein PAP with a tissue plasminogen activator preprosequence under control of the SV40 major late promoter. This was introduced into BHK by the Ca phosphate method. A transformant was used to produce the protein on a large scale. Amino acid sequencing of the affinity and gel-electrophoresis purified protein (appearing as two bands) confirmed the presence of both proteins in the fusion. The protein was fully active (sic) in amidolytic and anticoagulant activity.

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9109953	A1	19910711	WO 1990-US7335	19901213
W: AU, CA, JP				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, NL, SE				
US 5225537	A	19930706	US 1989-459082	19891229
AU 9170306	A1	19910724	AU 1991-70306	19901213

L29 ANSWER 10 OF 20 CAPLUS COPYRIGHT 2002 ACS

AN 1991:672705 CAPLUS

DN 115:272705

TI Recombinant activated **protein C** with truncated light chain

SO PCT Int. Appl., 50 pp.
CODEN: PIXXD2

IN Miyagi, Fuminori; Sumi, Yoshihiko; Wakabayashi, Kenji; **Foster, Donald C.**

AB An analog of activated human **protein C** with truncated light chain is produced with recombinant animal cells. Methods for purifying the protein using ion-exchange chromatog. and of detg. the ratio of the various forms of the recombinant **protein C** are described. The **protein C** analogs can be used in pharmaceuticals (no data). A gene for **protein C** with 2 addnl. arginine residues at the junction of the light and heavy chains is prepd. by site-specific mutagenesis. The mutant gene was expressed in BHK and 293 cells and the recombinant **protein C** was digested with thrombin and purified by chrpmatog. on S-Sepharose Fast Flow and Q-Sepharose Fast Flow columns. Activated **protein C** contg. light chains of amino acids 1-150, 1-151, and 1-152 were obtained.

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9112320	A1	19910822	WO 1991-US912	19910208
W: JP				
JP 05506354	T2	19930922	JP 1991-505079	19910208

L29 ANSWER 11 OF 20 CAPLUS COPYRIGHT 2002 ACS

AN 1991:672664 CAPLUS

DN 115:272664

TI Recombinant manufacture of **protein C** analogs with truncated light chains

SO PCT Int. Appl., 61 pp.
CODEN: PIXXD2

IN **Foster, Donald C.**; Holly, Richard D.; Suzuki, Masahiko; Wakabayashi, Kenji; Kumar, Anur Ashok

AB **Protein C** and analogs are manufd. by expression of the cloned gene in animal cells. The analogs contain modifications around the cleavage site for activation. Some of these analogs are cleavable by the KEX-2 proteinase of Saccharomyces cerevisiae allowing the manuf. of activated **protein C** in cells expressing the KEX-2 gene. A cDNA was cloned by antibody screening of a liver cDNA bank in .lambda.gt11. This was expressed in COS-1 and 293 cells using an SV40-based expression vector. Yields of **protein C** in the medium were 10 and 50 ng/mL for COS-1 and 293 cells resp. Over 70% of the protein was .gamma.-carboxylated. The cleavage site for activation was modified by insertion of basic amino acids with or without deletion of other amino acids. Yields of one of these analogs when the gene was expressed in BHK cells reached 2.2 pg/cell/day.

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9109951	A2	19910711	WO 1990-US7617	19901221
	WO 9109951	A3	19910822		
	W: JP				
	JP 05506353	T2	19930922	JP 1991-504341	19901221
	JP 3153236	B2	20010403		

L29 ANSWER 12 OF 20 CAPLUS COPYRIGHT 2002 ACS
AN 1991:672663 CAPLUS
DN 115:272663
TI Inhibition-resistant human **protein C** analogs containing peptides from bovine **protein C**
SO PCT Int. Appl., 66 pp.
CODEN: PIXXD2
IN **Foster, Donald C.**; Holly, Richard D.
AB Analogs of human blood-coagulation factor **protein C** that are resistant to inactivation by human plasma and .alpha.-1-antitrypsin are prepd. by exchanging selected peptides in human **protein C** with peptides from bovine **protein C**. These analogs are useful in the treatment of clotting disorders. An analog with the human heavy chain of activated **protein C** completely substituted by the bovine heavy chain was manufd. by expression of the corresponding cDNA in BHK cells. Under test conditions where human **protein C** was inhibited .apprx.60% by .alpha.-1-antitrypsin 800 .mu.g/mL the hybrid protein retained >90% of its activity. When exposed to human plasma the hybrid mol. retained .apprx.90% of its activity in 20% human plasma whereas human **protein C** retained only 20% of its activity. Analogs in which short peptides were replaced were also prepd.

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9109960	A1	19910711	WO 1990-US7693	19901228
	W: AU, CA, JP				
	RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, NL, SE				
	CA 2071630	AA	19910630	CA 1990-2071630	19901228
	AU 9171685	A1	19910724	AU 1991-71685	19901228
	EP 506821	A1	19921007	EP 1991-901991	19901228
	EP 506821	B1	19980204		
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE				
	JP 06502986	T2	19940407	JP 1991-502949	19901228
	JP 3270462	B2	20020402		
	AT 163048	E	19980215	AT 1991-901991	19901228
	ES 2113878	T3	19980516	ES 1991-901991	19901228
	US 5358932	A	19941025	US 1993-126440	19930923
	US 5766921	A	19980616	US 1994-318579	19941005
	US 5753224	A	19980519	US 1995-463585	19950605

L29 ANSWER 13 OF 20 CAPLUS COPYRIGHT 2002 ACS
AN 1991:606214 CAPLUS
DN 115:206214
TI Cell culture methods for producing activated **protein C**
SO PCT Int. Appl., 48 pp.
CODEN: PIXXD2
IN Kumar, Anur Ashok; **Foster, Donald C.**
AB A method for manufg. activated **protein C** comprises culturing mammalian cells stably transfected with an activated **protein C**-encoding expression vector in a culture medium contg. .ltoreq.0.1% serum and isolating the product. An expression plasmid encoding a protein consisting of the light chain and heavy chains of **protein C** linked by the tetrapeptide R-R-K-R (i.e. the activation peptide was deleted) was constructed. BHK cells stably transformed with this plasmid were prepd., and the transformant was further transformed with an expression plasmid for the KEX2 gene of Saccharomyces cerevisiae. When these double transformants were cultured in serum free media (also contg. vitamin K), more activated **protein C** was produced than when the cells were cultured in serum-contg. medium.

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE

PI WO 9102065 A1 19910221 WO 1990-US4419 19900807
W: AU, BB, BG, BR, CA, FI, HU, JP, KR, LK, MC, MG, MW, NO, RO, SD, SU
RW: AT, BE, BF, BJ, CF, CG, CH, CM, DE, DK, ES, FR, GA, GB, IT, LU, ML, MR, NL, SE, SN, TD, TG
CA 2064774 AA 19910212 CA 1990-2064774 19900807
AU 9062953 A1 19910311 AU 1990-62953 19900807
EP 485504 A1 19920520 EP 1990-912701 19900807
EP 485504 B1 19931201
R: AT, BE, CH, DE, DK, ES, FR, GB, IT, LI, LU, NL, SE
JP 04507347 T2 19921224 JP 1990-512234 19900807
JP 3045307 B2 20000529
AT 97958 E 19931215 AT 1990-912701 19900807
ES 2047946 T3 19940301 ES 1990-912701 19900807

L29 ANSWER 19 OF 20 CAPLUS COPYRIGHT 2002 ACS
AN 1997:457163 CAPLUS
DN 127:105237
TI Recombinant production in transgenic animals of **protein C** modified at cleavage site between light and heavy chains
SO PCT Int. Appl., 98 pp.
CODEN: PIXXD2
IN Garner, Ian; Cottingham, Ian; Temperley, Simon M.; **Foster, Donald C.**; Sprecher, Cindy A.; Prunkard, Donna E.
AB Methods for producing **protein C** in transgenic non-human mammals are disclosed. The **protein C** is modified at the two-chain cleavage site between the light and heavy chains of **protein C** from Lys-Arg to R1-R2-R3-R4 where R1 through R4 are individually Arg or Lys. DNA segments encoding modified **protein C** are introduced into the germ line of a non-human mammal, and the mammal or its female progeny produces milk contg. **protein C** expressed from the introduced DNA segments. The **protein C** expressed from the introduced DNA segments has anticoagulant activity when activated. Non-human mammalian embryos and transgenic non-human mammals carrying DNA segments encoding heterologous **protein C** are also disclosed.

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9720043	A1	19970605	WO 1996-US18866	19961126
W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9710246	A1	19970619	AU 1997-10246	19961126
EP 874898	A1	19981104	EP 1996-940607	19961126
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2000501928	T2	20000222	JP 1997-520589	19961126

L29 ANSWER 20 OF 20 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1999:356848 BIOSIS
TI **Protein C** production in non-human transgenic mammals.
SO Official Gazette of the United States Patent and Trademark Office Patents, (5/18/1999) Vol. 1222, No. 3, pp. NO PAGINATION.
ISSN: 0098-1133.
AU Garner, Ian (1); Cottingham, Ian R.; Temperley, Simon M.; **Foster, Donald C.**; Sprecher, Cindy A.; Prunkard, Donna E.
PI US 5905185

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